A Partial Failure of Membrane Protein Turnover May Cause Alzheimer’s Disease: A New Hypothesis

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Abstract: The amyloid hypothesis has dominated the thinking in our attempts to understand, diagnose and develop drugs for Alzheimer’s disease (AD). This article presents a new hypothesis that takes into account the numerous familial AD (FAD) mutations in the amyloid precursor protein (APP) and its processing pathways, but suggests a new perspective beyond toxicity of forms of the amyloid β-peptide (Aβ). Clearly, amyloid deposits are an invariable feature of AD. Moreover, although APP is normally processed to secreted and membrane-bound fragments, sAPPβ and CTFβγ, by BACE, and the latter is subsequently processed by γ-secretase to Aβ and CTFγ, this pathway mostly yields Aβ of 40 residues, and increases in the levels of the amyloidogenic 42-residue Aβ (Aβ42) are seen in the majority of the mutations linked to the disease. The resulting theory is that the disease is caused by amyloid toxicity, which impairs memory and triggers deposition of the microtubule associated protein, Tau, as neurofibrillary tangles. Nevertheless, a few exceptional FAD mutations and the presence of large amounts of amyloid deposits in a group of cognitively normal elderly patients suggest that the disease process is more complex. Indeed, it has been hard to demonstrate the toxicity of Aβ42 and the actual target has been shifted to small oligomers of the peptide, named Aβ derived diffusible ligands (ADDLs). Our hypothesis is that the disease is more complex and caused by a failure of APP metabolism or clearance, which simultaneously affects several other membrane proteins. Thus, a traffic jam is created by failure of important pathways such as γ-secretase processing of residual intramembrane domains released from the metabolism of multiple membrane proteins, which ultimately leads to a multiple system failure. In this theory, toxicity of Aβ42 will only contribute partially, if at all, to neurodegeneration in AD. More significantly, this theory would predict that focusing on specific reagents such as γ-secretase inhibitors that hamper metabolism of APP, may initially show some beneficial effects on cognitive performance by elimination of acutely toxic ADDLs, but over the longer term may exacerbate the disease process by reducing membrane protein turnover.

Keywords: Alzheimer’s disease, AICD, oligomers, dementia, neurodegeneration.

INTRODUCTION

The devastation of Alzheimer’s disease (AD) underscores the urgent need for accurate early diagnosis and treatment, making it a central focus of studies on neurodegeneration. A major problem in AD is the early loss of cholinergic function with loss of basal forebrain cholinergic neurons [1]. Since extensive neuronal cell loss is normally seen in AD when symptoms become apparent, treatment after the onset of symptoms may be quite difficult without neogenesis of neurons or a stem cell replacement program. Identification of the cause of neuronal dysfunction and death in the AD brain is hence of prime importance in order to prevent and treat the disease. A number of studies point to the amyloid β peptide (Aβ) deposited in the AD brain as a key culprit in AD pathogenesis [2-4]. These studies highlight the almost invariable association of amyloid plaques with clinically identified AD; identification of mutations on the amyloid precursor protein (APP) in rare familial forms of AD (FAD); demonstration that other FAD mutations on presenilins also increase the levels of the longer, 42-residue form of Aβ (Aβ42); discovery that apolipoprotein E (ApoE), a key risk factor in AD pathogenesis, is necessary for Aβ deposition [5]; identification of toxic forms of Aβ (soluble aggregates or oxidized)[6-8] that can cause neuronal cell loss; and association of clusterin with aggregation of Aβ and neurotoxicity [9]. Two parallel articles discuss the amyloid hypothesis with one by Lee et al. (This issue) significantly opposing the hypothesis and the second, by Hardy (This issue), discusses the opposition to the widely accepted amyloid hypothesis and points out that the best correlation of AD pathogenesis among all the mutations that are known to elicit the entire pathological spectrum of AD is Aβ42. Further, the article points out that normal objections to the amyloid hypothesis, such as the variability in Aβ toxicity, the poor correlation of plaque numbers or soluble amyloid with extent of dementia, fail to recognize the multiple forms of Aβ42, of which only...
one—the oligomer [3]—is likely to be important in pathogenesis. Moreover, this form may be transient, which will make the absolute correlation between levels of Aβ or amyloid plaques and dementia a poor measure of its role in the causation of AD. A similar controversy in the Tau protein, assigning a protective role to the precipitated neurofibrillary tangles. (NFTs) and accepts Aβ as a potential trigger.

This article attempts to bring together all these hypotheses and culminates them into an alternative theory. The authors feel that while it is imperative that drugs based on the amyloid hypothesis be tested, in light of the extensive correlations as well as animal model data supporting a direct toxic role for some form of amyloid in AD pathogenesis, it is equally important to recognize that the hypothesis is not yet proven and viable alternatives to the hypothesis need to be examined that could, in turn, lead to practical drug targets. Moreover, given the large drug development effort that already follows the amyloid hypothesis, alternative hypotheses need to be seriously considered. Indeed, as discussed in this review, the strongest arguments against the hypothesis are that Aβ42 is present in large quantities (~100 pM) even in the normal cerebrospinal fluid (CSF) that bathes the brain; suggesting that the protein is well tolerated by the neurons. Moreover, animal models with amyloid plaques do not show neurodegeneration, although careful analysis reveals the presence of behavioural deficits. However, even these findings contrast with the human disease, wherein dementia is linked to neuronal cell loss. The ultimate goal of a treatment program is to stop this progressive degeneration of neurons, making the identification of the mechanisms of degeneration critical.

AMYLOID HYPOTHESIS AND NEED FOR ALTERNATIVES

There is a large body of evidence in support of the amyloid hypothesis and a discussion of all the evidence is beyond the scope of this review but has been considered in an article in this issue by Hardy, as discussed above. Nevertheless, there are several inconsistencies with the idea that Aβ in one form or another is a unitary cause of AD, which also means that the sole therapeutic goal must be to eliminate the toxic form of Aβ. Some of these problems are discussed in parallel articles by Lee et al., and Hardy. Ultimately, the decisive proof of the hypothesis can only be obtained by successful treatment of AD patients through exclusive and specific removal of the offending peptide, bearing in mind that the approach to achieve this, rather than the hypothesis, may fail for one reason or another, as exemplified by the neuroinflammation caused by active vaccination against Aβ. Unfortunately, most of the current treatments using cholinesterase inhibitors (tacrine, donepezil, rivastigmine and galantamine) or an NMDA receptor antagonist, memantine, only show a moderate capacity to manage the disease symptoms, which continue to worsen with time and make treatment of the underlying cause an important priority. Despite long (~17 years) and intense efforts directed at establishing the amyloid hypothesis and the provocatively effective treatment of transgenic mouse models, drug development efforts based on the hypothesis have yet to yield a magic bullet that can arrest the disease in its tracks. Interestingly, behavioural deficits in transgenic mice can be arrested and even reversed by immunotherapy and other treatments, suggesting that the behavioural deficits in these animals are caused by amyloid aggregates. However, the field has stumbled on the unpredicted toxicity associated with several of these treatment strategies in human treatment trials and, thus far, their clinical benefits appear to be quite modest [10, 11]. Thus, the amyloid hypothesis for neurotoxicity has been proven in animal models but, to date, it falls short in the clinic.

A key concern is the failure to observe the duplication of the process of neurodegeneration in current animal models of AD, even though amyloid aggregates do form and deposit in transgenic mice expressing FAD mutant APP and acute amyloid-dependent CNS dysfunction and memory deficits are observed [12, 13]. Secondly, if oligomers of Aβ are only involved as transiently acting triggers of AD, treatment strategies that block their production, aggregation or toxicity may nevertheless fail, given that neurodegeneration is quite advanced before the onset of clinical symptoms.

It is important to note that the most frequent cause of FAD is mutations in a second protein, called presenilin 1 (PS1), rather than of APP [14]. A number of studies indicate that PS1 and its homologue, PS2, are the catalytic subunits of the final step in Aβ production — intramembrane cleavage of the APP transmembrane domain by a multisubunit enzyme named γ-secretase [14, 15]. Interestingly, FAD mutations in the PS1 protein normally increase levels of the longer 42-residue form of Aβ while effects on the more abundant 40-residue form are minimal. Although these studies provide a high degree of correlation of FAD mutations with Aβ42 and the latter with AD, it has not been directly demonstrated that AD is caused by Aβ42 and its aggregated derivatives. Thus, Aβ42 aggregates can be highly toxic to neuronal cell cultures, but are well tolerated by a number of cognitively normal patients with a high amyloid load as well as by mouse models expressing transgenic FAD mutant APP, which show behavioural deficits without neuronal loss. Several studies have also demonstrated that FAD mutations affect other γ-secretase substrates as well as other aspects of APP processing.

Thus, although support for the amyloid hypothesis is strong and it is important to develop drugs that reduce amyloid until the methods developed can safely clear these deposits, it is important to not lose sight of alternative hypotheses that can likewise explain the FAD mutations and to explore alternative mechanisms of neurodegeneration as a backup to or an extension of the amyloid hypothesis. In this regard, major advances may likely come from the detailed analysis of the mechanisms of neurodegeneration in the AD brain, as animal model alternatives for this process are not available and common molecular cascades may underpin other neurodegenerative diseases. Exploration and development of novel animal models may also be very important.

AMYLOID INDUCED NEURODEGENERATION

While searching for exceptions that either support or refute the amyloid hypothesis, we find a number of deviations that remain to be adequately explained.

1) Mutations in APP that frame Aβ are rare but mutations on presenilin are more common. Although both these muta-
tions share the property of increasing Aβ42, the age of onset of AD in the APP mutations are strongly influenced by the ApoE4 allele but the effects of this risk factor on the presenilin mutation is highly attenuated [16-18]. One explanation is that the presenilin mutations are more aggressive than APP ones, but the extent of the increase in levels of Aβ42 by the PS1 mutations is not consistent with the notion that the pathogenesis is now ApoE genotype independent.

2) There are some mutations in the middle of the Aβ sequence that actually result in a decrease in the levels of Aβ but still cause AD. Although this is explained by an increase in the tendency of the peptide to aggregate, this phenomenon has not been fully established in vivo [19, 20].

3) A rare mutation on PS1, insR352, does not show increases in Aβ42 production but inhibits γ-secretase and is associated with frontal temporal dementia, a condition associated with NFT formation [20, 21].

4) The Aβ sequence is highly conserved and the peptide is normally present at very high levels in the CSF. Thus, although a strong correlation has been observed between FAD mutations and increases in Aβ42, it is hard to explain the neurodegeneration in terms of the Aβ42 toxicity.

5) Down’s syndrome is often taken as an example of the importance of APP in neurodegeneration; however, the Down’s syndrome variants that lack APP are also associated with at least some neurodegeneration [22].

6) Mouse models of Down’s syndrome that fail to deposit amyloid show basal forebrain cholinergic neuronal loss. This type of neurodegeneration is characteristic of AD, but is not seen in mice over expressing FAD mutant APP to deposit amyloid. Thus, at least in this model, the neurodegeneration cannot be explained by Aβ42, its oligomers or amyloid deposits although duplication of APP appears to still be important [23].

7) AD is characterized by a multiple system failure as exemplified by the following: amyloid deposition as senile plaque and cerebrovascular amyloid [24], Tau deposition as NFTs [25, 26], ApoE fragmentation [27], Tau fragmentation [28, 29]; loss of white matter [30]; increase in selected endocytosis pathways such as autophagy [31, 32]; presence of other lesions such as the Lewy body [33, 34]; increased oxidative stress markers [35]; such as isoprostanes [36]; presence of DNA lesions [37]; increased molecular misreading [38]; presence of inflammatory markers such as IL-6, C-reactive protein, increases in LDL and HDL [16]; elevated homocysteine [39]; reduced sulfatides [40]; reduced neurotrophins, such as BDNF and NGF and their receptors [41]; and accumulation of Cu++ and Zn++ [42].

8) AD risk is also associated with a number of other diseases such as cardiovascular risk factors, type 2 diabetes, brain trauma, stroke, and these do not necessarily correlate with amyloid burden [43-48].

Fig. (1). Model for the failure of membrane recovery and recycling pathways upon failure of γ-secretase activity. Presenilin-dependent γ-secretase plays a role in the clearance of membrane-bound protein fragments (CTFX; e.g. CTFβ) generated by proteolytic release of the ectodomain of integral membrane proteins (X; e.g. APP, ApoER). Failure of the pathway results in the accumulation of secreted and cytoplasmic fragments of non-physiological size or subcellular localization. This not only compromises signalling steps (e.g. Notch), but also prevents waste removal, membrane lipid turnover and nutrient delivery that causes cellular dysfunction and ultimately death.
9) BACE overexpression leads to neuronal dysfunction, despite a reduction in the levels of Aβ [49, 50]

ALTERNATIVE HYPOTHESIS

In this article, we present an alternative hypothesis for AD pathogenesis. The hypothesis states that AD is caused by a multi-hit mechanism. We and others have previously defined γ-secretase as a multicatalytic proteosome-like enzyme, secretosome uniquely designed to eliminate membrane protein remnants, which are normally protected from other housekeeping proteases, and propose that a partial failure of this activity initiates the process (Fig. 1) [16, 51-53]. This will result in the accumulation of a number of substrates in the membrane — ultimately causing a failure of alternative backup pathways for protein turnover in the membrane, accumulation of proteins near the synapse leading to failure of membrane recycling and recovery in this important and active location. This disruption is also reflected in the cellular cholesterol uptake and recirculation, which then leads to failure of cholesterol homeostasis. This disruption of the normal cellular trafficking pathways leads to accumulation of Tau in the cells, which in turn causes a failure of microtubules and degeneration of the synapse (Fig. 2). The process may be further aggravated by an attempt to accumulate cholesterol to restore some of the synaptic plasticity in these dysfunctional neurons. Consistent with this model, free cholesterol was found differentially accumulated in tangle-bearing neurons and failure of cholesterol homeostasis in Niemann Pick C disease leads to formation of NFTs [54, 55]. Under such circumstances, Aβ and Tau are the accumulated proteins readily detected by their detergent insoluble deposits and because of their high expression levels, constitutive production and tendency to aggregate and deposit. Indeed, this second step of Aβ accumulation represents a failure in the cellular capacity to regulate its production due to impairment of γ-secretase as well as a second clearance pathway of the soluble protein, which may also lead to accumulation of other proteins and metabolites. In fact, aggregation and deposition may be a mechanism that serves as a backup pathway to eliminate the excess proteins due to the failure of the normal clearance pathways and therefore serve as an excellent marker for the disease. The removal of Aβ or reduction in its production may have a modest early effect in arresting the progression of AD by providing relief to the second clearance pathways and declogging the system, but inhibition of the proteases that generate Aβ, particularly γ-secretase, may have undesirable long-term consequences due to accumulation of APP CTFα and CTFβ and other similar membrane-bound protein metabolites, even if the inhibitors do not affect the function of a developmentally important substrate. Notch. The normal improvement in behaviour associated with acute removal of amyloid after immunotherapy of transgenic mice, could presumably be due to reduction in the levels of ADDLs or amyloid deposits [56]. Numerous behavioural deficits are also reported to be associated with amyloid deposits although some of these deficits predate amyloid plaque formation and may therefore be independent of amyloid deposits and caused by the oligomers [57]. The acute toxicity of ADDLs in these mice that express enormous amounts of APP, may be readily corrected by the removal of Aβ. However, the same may not be true in humans, who express normal levels of APP and accumulate a complex of proteins including Aβ. The mental deficits here may be caused by neuronal dysfunction and loss caused by failure of the metabolism of multiple proteins and effective treatment strategies may need to be more focussed towards improving clearance of all these proteins by fostering multiple turnover pathways, such as plasmin and nephrilysin. In addition, it likely will be imperative to control the collateral damage caused by inflammatory pathways that are activated as the body’s alternative clearance mechanisms to remove the protein deposits and dysfunctional cells.

MULTIPLE TOXIC MECHANISMS IN AD

A major difference between the novel hypothesis presented herein and other alternative theories in the field is that it acknowledges the potential role for Aβ. However, it also recognizes a number or complex events, some of which are also slowly being established as signatures of AD, and attempts to bring together the diverse theories on the pathogenesis of the disease. These include early cognitive deficits that appear to be associated with people who eventually develop AD [58-60], detection of fragmented apolipoprotein E that is also toxic and induces neurofibrillary degeneration [27], genetic linkage of the apoE receptor—LRP—with AD [61], induction of tangle formation by proteolytic cleavage of Tau [28], identification of PS1 mutations that fail to increase Aβ42 but induce Tau deposits [20], toxicity of fragments derived from the cytoplasmic domain of APP [62, 63], detection of defective mitochondria in platelets of AD patients [64, 65] and impairment of neurotransmission due to the loss of white matter in the AD brain [30]. It tries to reconcile the finding that amyloid-induced behavioural deficits are distinct from AD, where the behavioural deficit is not linked to levels of amyloid aggregates but with progressive neuronal loss and NFTs and failure of cholesterol homeostasis. Thus, the major deviation from the amyloid hypothesis relates to the type of neuronal dysfunction and loss. The amyloid hypothesis relies on acute toxicity of Aβ oligomers, while this hypothesis proposes that the failure of membrane recycling near the synapse triggers a compensatory response to deliver more cholesterol and phospholipids down the axon. The additional accumulation of protein leads to even greater accumulation of partially processed membrane proteins. While there is an attempt to clear these accumulated proteins and membrane vesicles by increasing phagocytosis, the pathways of clearance are overwhelmed and a large accumulation of debris, combined with failure to deliver nutrients, leads to starvation and smothering of the neurons. The specific pathways involving failure from the axon end results in accumulation of Tau inside the cell, in the nonphysiological somato-dendritic compartment, which precipitates as tangles. Accumulation of these precipitated proteins may not, per se, cause the cell death, but are part of the process of trafficking failure, with cells remaining alive for a long time in a dysfunctional state and the more susceptible cells dying even though they may not contain any tangles or plaques. Additional toxic mechanisms such as those caused by inflammation during clearance of the debris and toxicity of ADDLs may be superimposed on this underlying failure to recycle cellular components.
Fig. (2). Model showing neurodegeneration triggered by failure of APP processing at the synapse: In neurons, BACE (1) is transported to the dendrite (A) and α-secretase (2) to the axon (B), while γ-secretase (7) is in both compartments. APP (3) is trafficked primarily to the axon and slightly to the dendrite, which limits its processing by BACE [112]. The microtubule-binding protein, Tau (10) is localized in the axon bound to microtubules (11) as shown in panel B. Most APP is processed by α-secretase, in response to signalling events in the synapses to membrane-bound CTFα (4) and secreted sAPPα (5). The membrane bound CTFα and small amounts of CTFβ are turned over by γ-secretase to release P3 and Aβ peptides into the synaptic cleft and CTFγ into the cytoplasm. The released CTFγ is normally turned over rapidly before it can reach the nucleus. Panels C and D delineate the events in the somatodendritic and Axon-synaptic compartments of degenerating neurons in the AD brain as predicted by the presented hypothesis. Impairment of γ-secretase processing results in the accumulation of vesicles containing CTFα and impairs recycling of these vesicles. The resulting traffic jam disrupts microtubule-mediated rapid transport and the microtubule-Tau interaction leading to accumulation and aggregation of the axonal proteins such as Tau in the cell body. APP now accumulates in the somatodendritic compartment and the CTFγ released enters the nucleus to induce GSK3β (12) expression, which then phosphorylates Tau.

PRESENILIN-RELATED CHANGES SUGGEST A PROTECTIVE ROLE OF γ-SECRETASE IN AD

1) Several studies have indicated that APP may have more than one toxic domain. A second toxic domain associated with apoptosis is a 3 kDa fragment of APP generated by caspase cleavage [62].

2) It has been shown that partial inhibition of γ-secretase by transition state inhibitors or by antisense RNA to PS1
results in an increase in Aβ42 [66, 67]. This sparked a lively debate on whether presenilin mutations represented dominant negative functions or a dominant gain of new function. During this period, it was demonstrated that presenilin mutations displayed a diminished ability to complement a loss of function mutation of Sel 12 in C. elegans [68, 69].

3) More recently, studies have shown that several presenilin mutations linked to FAD result in a reduced capacity to cleave APP to generate CTFγ (AKA: AICD, AID, Cγ, CTFε), a 50 amino acid fragment consisting of the APP intracellular domain and three residues of its transmembrane domain [70-73]. In addition, mis-processing was observed in at least some FAD mutations, which yielded an alternative CTFγ fragment of 51 residues. Moreover, the complementary Aβ48 fragment preferentially generated Aβ42 after further processing by γ-secretase [74, 75]. The function of the CTFs of APP are not fully identified but the region is highly conserved and has been shown to influence trafficking [76] and transcription [77-80].

4) Failure of γ-secretase processing of other substrates, such as Cadherins, has also been observed as a consequence of FAD mutant presenilins [81, 82]. There is a large and growing list of proteins identified as γ-secretase substrates (Table 1).

5) Deficiencies in presenilin using a conditional knockout mouse show deficiencies in behaviour and neurodegeneration [83].

6) Partial loss of APH1 due to gene rearrangement was recently found to be linked to the phenotype of an apomor-

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<td>Lipoprotein receptor, neuronal migration</td>
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<td>APP</td>
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Faulty Protein Turnover May Cause AD

In short, while there is a strong correlation between APP, presenilins, ApoE and AD, and there is strong support for the hypothesis that Aβ is one of the triggers and that its accumulation in non-physiological aggregated forms may be harmful. Nevertheless, despite the findings that Aβ aggregates can be toxic, the neurodegeneration in AD cannot be explained by this simple unitary toxicity result of Aβ aggregates. Moreover, the best predictor of the MMSE score in AD has been reported to be the loss of neurons from the CA1 field of the hippocampus, entorhinal cortex, and area 9, which in turn, correlates with the NFTs load but is not incrementally affected by the amyloid burden [87, 88]. A more comprehensive hypothesis is that the neurodegeneration in AD is due to a slow accumulation of a number of membrane-bound and secreted-proteins consequent to the failure of mechanisms for their turnover and clearance. This article proposes that the process may be triggered by faulty interactions, processing and turnover of proteins, primarily γ-secretase substrates, facilitated by presenilins. Since APP is a highly expressed protein with a short half life, it is likely to be a major γ-secretase substrate and may influence the processing of the other substrates, particularly in its FAD version. The accumulation of all these substrates could create a ‘traffic jam’ in the membranes, which may be handled by alternative pathways such as stimulated endocytosis. Ultimately, there is a multiple system failure that leads to progressive neuronal cell death and dementia, which is triggered by an initial accumulation of partially degraded membrane proteins due to failure of clearance mechanisms.

Supporting the hypothesis, we have significant literature showing that partial failure of proteolysis is detrimental in a number of cases.

1) Parkinson’s disease shows deficiencies in ubiquitin-mediated proteolysis [89].

2) Neuronal ceroid lipofuscinoses (NCLs) are a group of lysosomal storage disorders characterized pathologically by neuronal accumulation of autofluorescent storage material and neurodegeneration, and are known to be caused by the loss of cathepsin D [90].

3) Mutant APP that cannot be processed by alpha secretase results in transgenic mice that show dominant progressive neurodegeneration, but APP transgenic mice that overproduce and deposit amyloid do not show signs of neurodegeneration [91].

Caveats

While the current hypothesis attempts to explain the complex pathology of AD along with the central genetic role demonstrated for APP, this theory is truly in the early stages of development with a number of unanswered questions. First, it is not known whether C-terminal membrane-bound fragments of APP and other γ-secretase substrates are accumulated early in the development of AD. Second and more importantly, there are a few mutations in the APP that are mapped to the middle of the Aβ sequence and do not appear to affect the levels of Aβ. However, the Aβ generated by these mutations show a greater tendency to aggregate, which supports the amyloid hypothesis [19]. It is possible that Aβ is the major culprit in this family, but even here it may be acting by impairing the general clearance of a number of proteins. Another caveat is that the complex processes are relatively poorly defined, making this hypothesis somewhat escapist. One may expect to see a defined series of events, given that AD consistently follows a pattern of cholinergic neuronal loss followed by more global changes. However, this theory is not truly random, but predicts a specific role for APP and similar proteins, including the lipoprotein receptors such as LRP that are transported down the axon towards the synapse. The cell loss is due to a chronic failure to supply essential nutrients as well as eliminate waste, which is not necessarily toxic, but smoothers the system, starting at the synaptic terminals.

Testable Predictions

The major value of a hypothesis is to identify testable predictions that can either prove or disprove a hypothesis. However, in a complex disease like AD, this is likely to be very difficult and we may have to settle for predictions that will strengthen or weaken the hypothesis. Based on our hypothesis, we predict the following:

1. Membranes from the degenerating neurons should reveal an accumulation of CTFs of APP that are γ-secretase substrates in the AD brain.

2. Fragments similar to APP CTF derived from Notch and other γ-secretase substrates (Table 1) should also accumulate in the AD brain around degenerating neurons.

3. FAD mutations should affect several membrane recycling processes, including cholesterol homeostasis.

4. Chronic sublethal inhibition of γ-secretase should result in neurodegeneration and possibly AD type pathology with hyperphosphorylation of Tau.

Reduced γ-secretase activity should be detected in affected neurons derived from the AD brain before overt degeneration.

An important conclusion of this hypothesis is that the treatment of AD cannot focus on elimination of just the Aβ deposits or countering Aβ toxicity. Instead, one has to improve the clearance mechanisms in the brain. This may be partially achieved by stimulating signal transduction pathways and α-secretase cleavage but would also need to be supported by improving other clearance pathways.
STATEMENT

We have consulted for and have some grant support from companies but have no competing financial interests. We observe ethical standards for responsible conduct regarding scientific communication.

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